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Tailoring liquid crystalline lipid nanomaterials for controlled release of macromolecules

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ABSTRACT

Lipid-based liquid crystalline materials are being developed as drug delivery systems. However, the use of these materials for delivery of large macromolecules is currently hindered by the small size of the water channels in these structures limiting control over diffusion behaviour. The addition of the hydration-modulating agent, sucrose stearate, to phytantriol cubic phase under excess water conditions incrementally increased the size of these water channels. Inclusion of oleic acid enabled further control of swelling and de-swelling of the matrix *via* a pH triggerable system where at low pH the hexagonal phase is present and at higher pH the cubic phase is present. Fine control over the release of various sized model macromolecules is demonstrated, indicating future application to controlled loading and release of large macromolecules such as antibodies.

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1. Introduction

Compared to small molecule therapeutics, the large size and complex structure of biological drugs such as antibodies and proteins present challenges in developing controlled release systems. Achieving longer duration release is also often more critical for these drugs due to their typical short half-lives and need for parenteral administration. Their size in particular can be problematic in achieving fine control over diffusion controlled release as tailoring pores to an appropriate dimension in polymeric materials requires a kinetically controlled process, and changes to the pore size during release can complicate release kinetics.

Lipid-based liquid crystalline materials are receiving increased attention as controlled release delivery systems for macromolecular drugs. Liquid crystal structures are formed by the self-assembly of certain amphiphilic lipids in an excess aqueous environment (Boyd et al., 2009, 2007; Fong et al., 2009; Kaasgaard and Drummond, 2006). Commonly used lipids are phytantriol (PHYT) and glycerol monooleate (GMO). The nanostructure that is formed is determined by the geometric packing of the lipid, defined by the critical packing parameter, expressed in Eq. (1) (Israelachvili, 1994).

$$\mathrm{CPP} = \frac{v}{a_o l} \quad (1)$$

where v is the hydrophobic chain volume, a_o is the surface area occupied by the hydrophilic head group area and l is the hydrocarbon chain length (Israelachvili, 1994).

The effective critical packing parameter is also influenced by factors such as the presence of additives, pH, temperature and pressure.

The liquid crystalline structures of typical interest for drug delivery purposes are the reverse bicontinuous cubic phase (V_2) and reverse hexagonal phase (H_2) (Yaghmur and Glatter, 2009). Hexagonal phase consists of infinite water rods arranged in a two-dimensional hexagonal lattice, separated by the lipid domain. The reverse bicontinuous cubic phase consists of two continuous water channels separated by a lipid bilayer (Boyd et al., 2007; Yaghmur and Glatter, 2009). Three commonly formed variants of the reverse bicontinuous cubic phase are the gyroid (Ia3d), diamond (Pn3m) and primitive (Im3m). The different reverse cubic and hexagonal structures are summarised schematically in Fig. 1 (Mulet et al., 2013).

When small molecules such as drugs are incorporated into these materials they have been shown to exhibit a diffusion controlled release profile where incorporated drug diffuses through the aqueous channels in order to escape to the continuous

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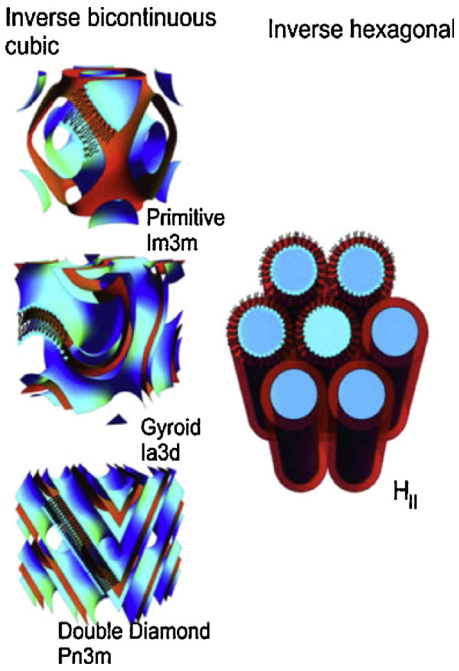

Fig. 1. Schematic representation of inverse bicontinuous cubic phase (V_2) of different space groups and reverse hexagonal phase (H_2 or H_{II}). Reproduced with permission from Mulet et al. (2013).

aqueous medium (Zabara and Mezzenga, 2014). Phan et al. showed that the release of small molecule drugs from the cubic phase is at a considerably faster rate than the other mesophases (Phan et al., 2011). The difference in release rates allows for an on-off on-demand release behaviour that would be particularly useful for pulsatile release of large macromolecules with short plasma half-lives. However, as a consequence of the limiting water channel size, release of large macromolecules is very slow (Lee et al., 2009). A means of loading larger hydrophilic molecules and facilitating triggered release would be advantageous.

There are strategies to increase the channel size in cubic phases, to make them more amenable to host and release macromolecules. The addition of sucrose stearate (ST), a sugar amphiphile with a very large hydrophilic headgroup, increases the average area of the head group at the lipid-water interface (a_o) decreasing the CPP value, thereby inducing a change in spacegroup from Pn3m to Im3m. The Im3m phase possesses a less negative curvature and a greater water intake capacity, increasing the size of the water channels. The addition of ST induced a concentration-dependent 4-fold increase in the size of water channels in the monolinolein-based cubic phase (Negrini and Mezzenga, 2012). It has also been reported that for GMO-water systems, oleic acid (OA) played a significant role in altering the phase behaviour. OA affects the molecular packing of the lipid, such that when the head group is deprotonated, electrostatic repulsion induces the formation of larger water channels (Du et al., 2014; Salentinig et al., 2010).

It is hypothesized in this study that OA has the potential to work synergistically with ST as additives to create a cubic phase with even larger water channels. Additionally, OA as an ionizable compound allows dynamic switching between H_2 and V_2 phases using pH (Du et al., 2014) and hence, has the potential to provide a mechanism to control drug release on demand. pH changes arise naturally within the human body, which makes this parameter an ideal stimulus (Kost and Langer, 2012; Langer, 1990; Negrini and Mezzenga, 2011). Specifically, at low pH, such as in the gastric compartment or in a precursor formulation the macromolecule would be trapped in the hexagonal phase when the water channels are narrow, inhibiting or preventing release, and then upon a change to typical physiological conditions in the intestine or subcutaneous space at neutral pH there would be a phase transition to a highly swollen cubic phase that triggers the release of the macromolecule in a controlled manner. Reversing the pH should induce a switch back to the previous structure and consequent drug release behaviour. Therefore, in order to test these hypotheses the impact of ST and OA on the phase behaviour of phytantriol in excess water was determined and the pH-dependent release of model macromolecular drugs of increasing size was determined to map the formulation space for on demand triggered release systems.

2. Materials and methods

2.1. Materials

Phytantriol was purchased from DSM (Singapore). Oleic acid was obtained from Croda International (Yorkshire, UK). Sucrose stearate was purchased from Mitsubishi-Kaguku Foods Corporation, Tokyo, Japan. These ingredients were used without further purification. Fluorescein isothiocyanate-dextrans (FITC-dextrans) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) with different molecular weights (4000 Da, 7100 Da, and 21,000 Da). Disodium hydrogen orthophosphate anhydrous, was purchased from APS Ajax Finechem (Auburn, NSW, Australia). Potassium dihydrogen orthophosphate, sodium chloride, citric monohydrate and trisodium citrate dihydrate were from BDH AnalaR, Merck Pty., Ltd. (Kilsyth, Victoria, Australia). The water used was obtained from a Millipore water purification system using a QuantumTM EX Ultrapure Organex cartridge (Millipore, Australia).

2.2. Preparation of bulk phase liquid crystals

The bulk liquid crystalline systems were prepared by combining phytantriol, oleic acid, sucrose stearate and FITC-dextran at different proportions, to 500 mg, in excess ethanol in a 20 mL glass scintillation vial. The mixture was vortex mixed and kept under vacuum at 40 °C for over 24 h to remove the ethanol. Phosphate buffered saline (pH 7.4) or citrate (pH 5.5) buffer at 1× concentration (10 mL) were then added to the vial and left to equilibrate at room temperature for 5–7 days. The pH of the buffer was monitored daily and adjusted using 1 M NaOH and HCl when required to maintain the pH.

2.3. Small angle X-ray scattering (SAXS)

The internal structure of the liquid crystalline systems was characterized using small angle X-ray scattering (SAXS) (Kirby et al., 2013). The equilibrated samples were loaded into a 96 well plate for high throughput structure determination. A custom-designed plate holder was used to mount the plate directly onto the SAXS/WAXS beamline. Scans were automated using a pre-loaded set of position variables based on the well positions within the plate.

Measurements were performed on the SAXS/WAXS beamline at the Australian Synchrotron (Kirby et al., 2013). An X-ray beam with a wavelength of 1.1271 Å (11 keV) was selected. The 2D SAXS patterns were collected using a Pilatus 1 M detector (active area $169 \times 179\,mm^2$ with a pixel size of 172 μm) which was located 966 mm from the sample position. The total q range for the instrument configuration outlined above was $0.016 < q < 1.06\,Å^{-1}$. Computer software, Scatterbrain, was used to acquire and reduce 2D patterns to 1D intensity *vs.* q profiles. Phase structures were identified by indexing Bragg peaks to known relative spacing ratios and lattice dimensions, a, calculated using known relationships (Hyde, 2012).

The lattice parameter, the dimensions of the repeating unit cells, which is obtained from the scattering data can be used to calculate water channel size. The channel radius was calculated using the following equations (Briggs et al., 1996):

$$(\mathrm{Pn3\,m}) \quad r = 0.391a - 1 \tag{2}$$

$$(\mathrm{Im3\,m}) \quad r = 0.305a - 1 \tag{3}$$

where r = the radius of the water channel (in nm), a = the lattice parameter (in nm) (determined *via* SAXS) and l = lipid chain length (in nm) from (Briggs et al., 1996).

To calculate the water channel radius from the hexagonal phase lattice parameter, the following equation was used based on geometric considerations:

$$r = \frac{(a - 2l)}{2} \tag{4}$$

2.4. In vitro *release experiment design and analysis*

A custom designed sample holder (Fig. 2) was used for the *in vitro* release studies. The sample holder contains 24 micro wells, which allows for a relatively high throughput release format under identical conditions.

Approximately 200 mg of liquid crystalline sample containing FITC-dextran was loaded into individual micro wells and then inverted into a clear 24 well plate, containing 2 mL of citric buffer (pH 5.5) in each well as release medium taking care to avoid the presence of trapped air bubbles. The plate was stored at 37 °C and placed on a Ratek Solid State Control agitator at 50 RPM.

The quantity of FITC-dextran released into the buffer was measured using a FLUOstar OPTIMA platereader (BMG Lab technologies, Germany). The concentration of FITC was recorded at an excitation and emission wavelength of 490 nm and 525 nm respectively. Aliquots of release medium (200 μL) were collected into a 96-well plate at regular time intervals and the fluorescence intensity measured at 37 °C. Fresh buffer (2 mL) was added to the sample well after each sample collection in order to maintain sink condition. Fluorescence intensity was compared to a standard curve to calculate percentage of FITC-dextran released over time.

The release medium was alternated between citrate buffer (pH 5.5) and PBS buffer (pH 7.4) every 5–7 days to observe any changes in release behaviour due to the influence of pH on matrix nanostructure.

The data for each of the triplicate release profiles for each phase was converted to percentage of FITC-dextran released against $t^{1/2}$ (h). The data showed a linear relationship against $t^{1/2}$ indicating diffusion controlled release, and the diffusion coefficient for each system was obtained using equation (Ho et al., 1972).

$$Q = 2C_o\left(\frac{Dt}{\pi}\right)^{1/2} \tag{5}$$

where Q is the amount of drug released per unit area of matrix, D is the diffusion coefficient of the drug in the matrix, C_o is the initial loading concentration in the matrix and t is time.

The mean of the diffusion coefficients for each of the three replicates for each system was taken and the values were analysed by one-way analysis of variance (ANOVA), with significance at a p-value < 0.05, to determine the significance between the release rates for the different liquid crystalline phases.

Fig. 2. Custom designed multi-sample holder used for the FITC-dextran release study. The wells are loaded with the liquid crystalline matrix and the entire holder inverted into the wells of a 24-well microplate on a shaker for high throughput release studies.

3. Results

3.1. Effect of additives and pH on phase behaviour

Previous studies have shown that addition of 10% OA to phytantriol cubic phase will produce a system that switches from cubic phase to hexagonal phase upon decreasing the pH from 7.4 to 5.5 (Du et al., 2014). Hence this was the starting point for these investigations. The scattering profiles in Fig. 3A illustrate that in the absence of ST at pH 7.4 the relative positions of the six Bragg peaks are in the ratio $\sqrt{2}:\sqrt{3}:\sqrt{4}:\sqrt{6}:\sqrt{8}:\sqrt{9}$ which are characteristic of the V_2 phase, while at pH 5.5 (Fig. 3B), three strong Bragg peaks were observed in the scattering profiles with the spacing ratio $1:\sqrt{3}:\sqrt{4}$, which corresponds to the H_2 phase. The inclusion of increasing proportions of ST did not alter the identity of the phases formed as the same peak patterns were evident, however an increase in the lattice parameter was observed with increasing ST concentrations, evidenced by shifting of the peak positions to lower q values—this effect is discussed in detail in Section 4. The data obtained at pH 5.5 are slightly compromised in peak shape due to the integration being impacted by the crystallinity of the samples, nevertheless the trends in lattice parameter are clear. At pH 5.5 on addition of 5% OA and 0% OA the system was in the cubic phase as expected.

The effect of increasing sucrose stearate concentration on the lattice parameter calculated from the scattering profiles for the phytantriol + 10% OA systems at pH 5.5 and 7.4 is summarized in Fig. 4. Increasing the ST concentration from 0 to 30% (w/w) induced a steady increase in lattice parameter, from 85 Å to 164 Å at pH 7.4 and from 47 Å to 95 Å at pH 5.5 (Fig. 4A). The sizes of the water channels were calculated for the V_2 and the H_2 phase from the lattice parameters determined using SAXS. For the 10% OA sample, the increasing size of the water channels increased with ST concentration logically following the behaviour of the lattice parameter.

Having established that the behaviour at 10% (w/w) OA and addition of ST provided substantial increases in the water channel diameter, and that the systems were pH responsive, the effect of changing the concentration of OA was determined to evaluate the full formulation space. The contour profiles in Fig. 5A for systems at pH 7.4 indicate that increasing oleic acid content up to 25% (w/w) had a similar effect on increasing the lattice parameter as addition of 30% ST, and that addition of both additives led to a synergistic increase in lattice parameter. The maximum change observed here was a fairly dramatic increase in lattice parameter from approximately 70 Å to close to 200 Å.

Interestingly, for the systems at pH 5.5 in Fig. 5B, the impact of increasing the concentration of OA was negligible or negative, and even appeared to inhibit the swelling of the hexagonal phase due to addition of ST especially at higher concentrations of OA. This is

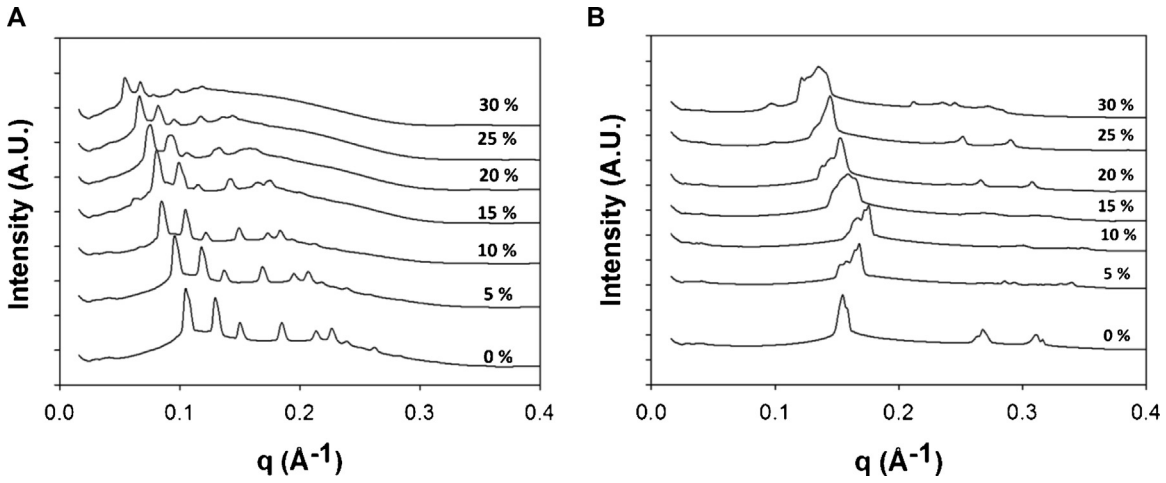

Fig. 3. Scattering profiles for PHYT-based liquid crystalline systems with [OA] fixed at 10% (w/w) and addition of up to 30% (w/w) ST at A) pH 7.4 and B) pH 5.5.

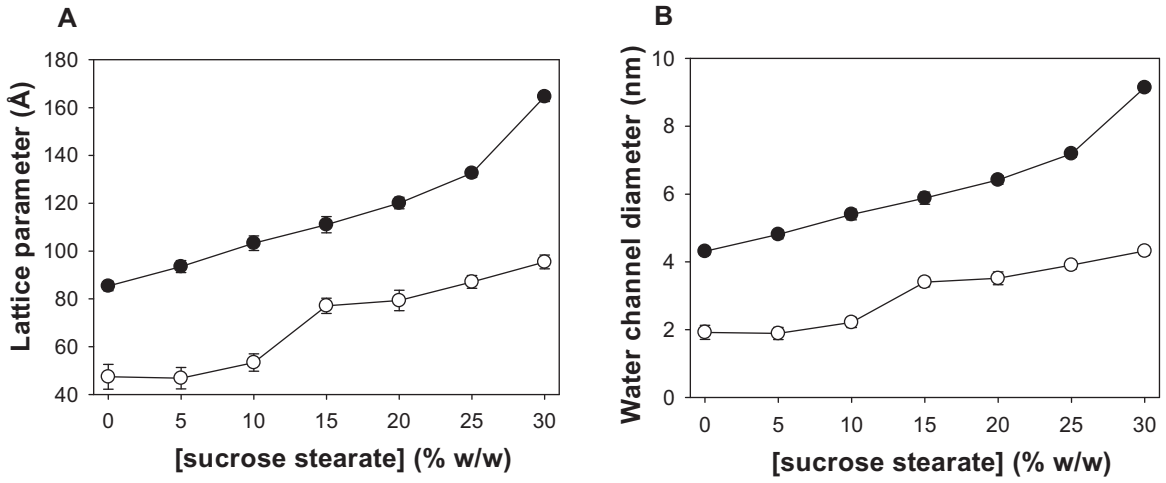

Fig. 4. Effect of addition of ST on structure of PHYT-based liquid crystalline systems at fixed [OA] = 10% (w/w) derived from scattering profiles in Fig. 4. Panel A. Lattice parameter at pH 5.5 (white circles) and pH 7.4 (black circles). Panel B. Water channel diameter derived from lattice parameter at pH 7.4 in cubic (V_2) phase (black circles) and pH 5.5 in hexagonal (H_2) phase (white circles). Error bars indicate the error in determination of the lattice parameter from peak positions in SAXS profiles (mean ± s.d, n = 3–4).

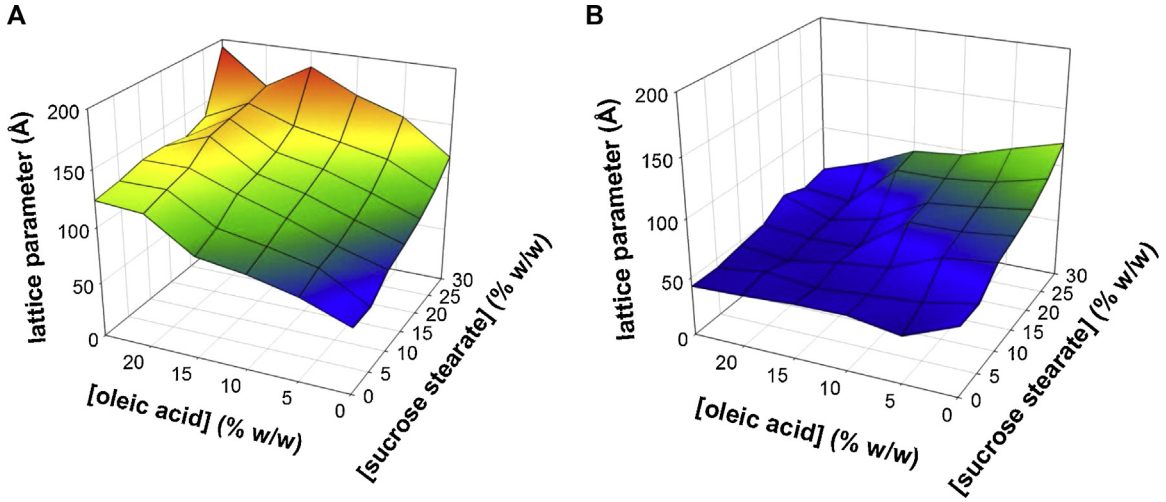

Fig. 5. Effect of additives on the lattice parameter of phytantriol-based liquid crystalline systems in excess aqueous solution with increasing concentrations of OA and ST. Panel A: changes in lattice parameter at pH 7.4 where samples are in the cubic phase region. Panel B: changes in lattice parameter at pH 5.5 where samples are hexagonal phase.

Table 1
Lipid composition (w/w), lattice parameter and the diameter of the water channels of the liquid crystalline structures used in the release study.

Composition			Lattice parameter (Å)	Water channel diameter (nm)
PHYT	OA	ST		
55	15	30	185.1	10.4
65	20	15	114.7	6.1

understandable due to the protonation of OA at the low pH leading to its inclusion in the hydrophobic volume of the hexagonal phase, essentially causing a negative swelling effect elaborated on in the discussion. The complete SAXS profiles and the lattice parameter of all systems studied are summarised in the supplementary section (Tables S1 and S2).

3.2. In vitro *release study*

In this study, three different sized FITC-dextrans, representing three model macromolecules were tested in two different lipid systems with large and small water channel sizes to confirm the impact of channel size on diffusion, and to evaluate the impact of changing pH and hence nanostructure. The composition of the systems and water channel size used in the study are summarised in Table 1.

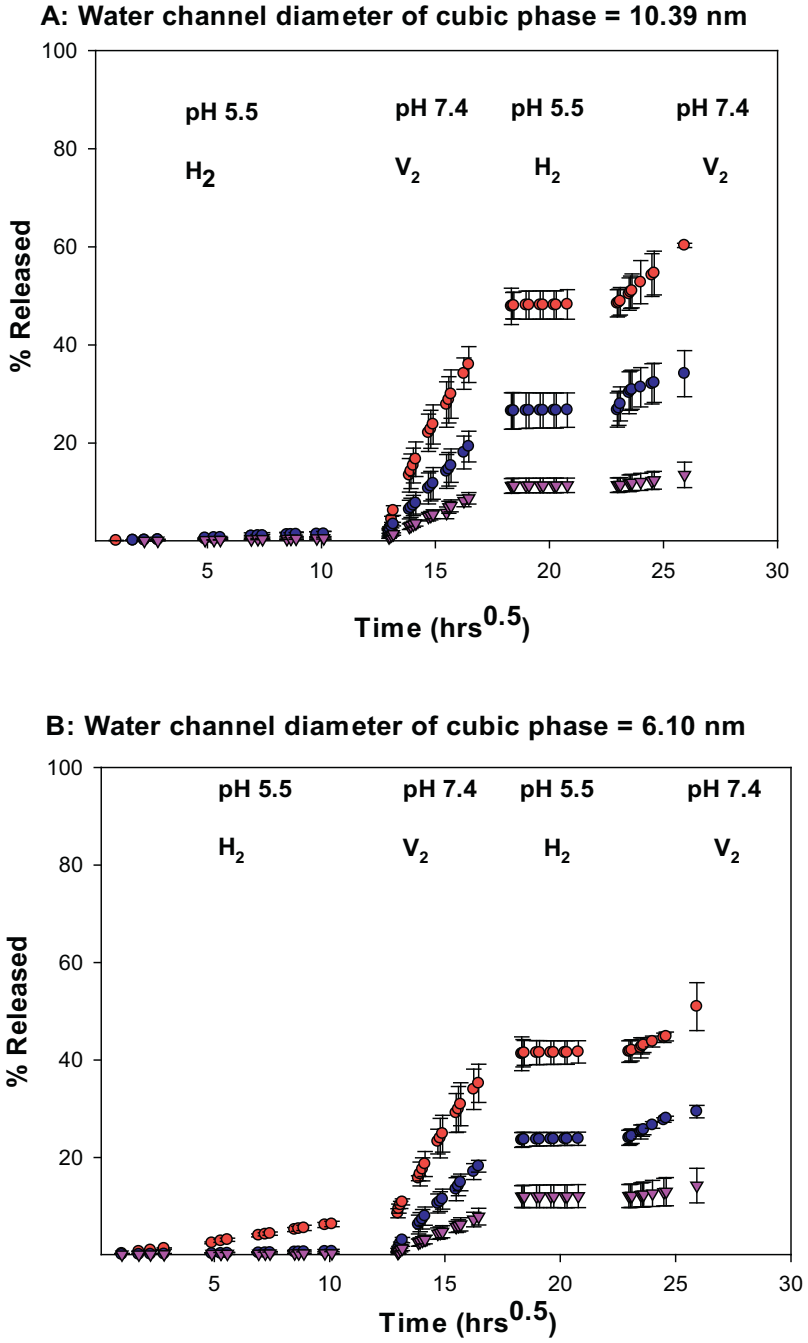

Fig. 6. Changes in phase and consequent changes in release rate triggered by pH for different water channel radius systems and size of FITC-dextran (red circles = 4000 Da, blue circles = 7000 Da and pink triangles = 21000 Da. (Data are mean ± s.d, $n = 3$).

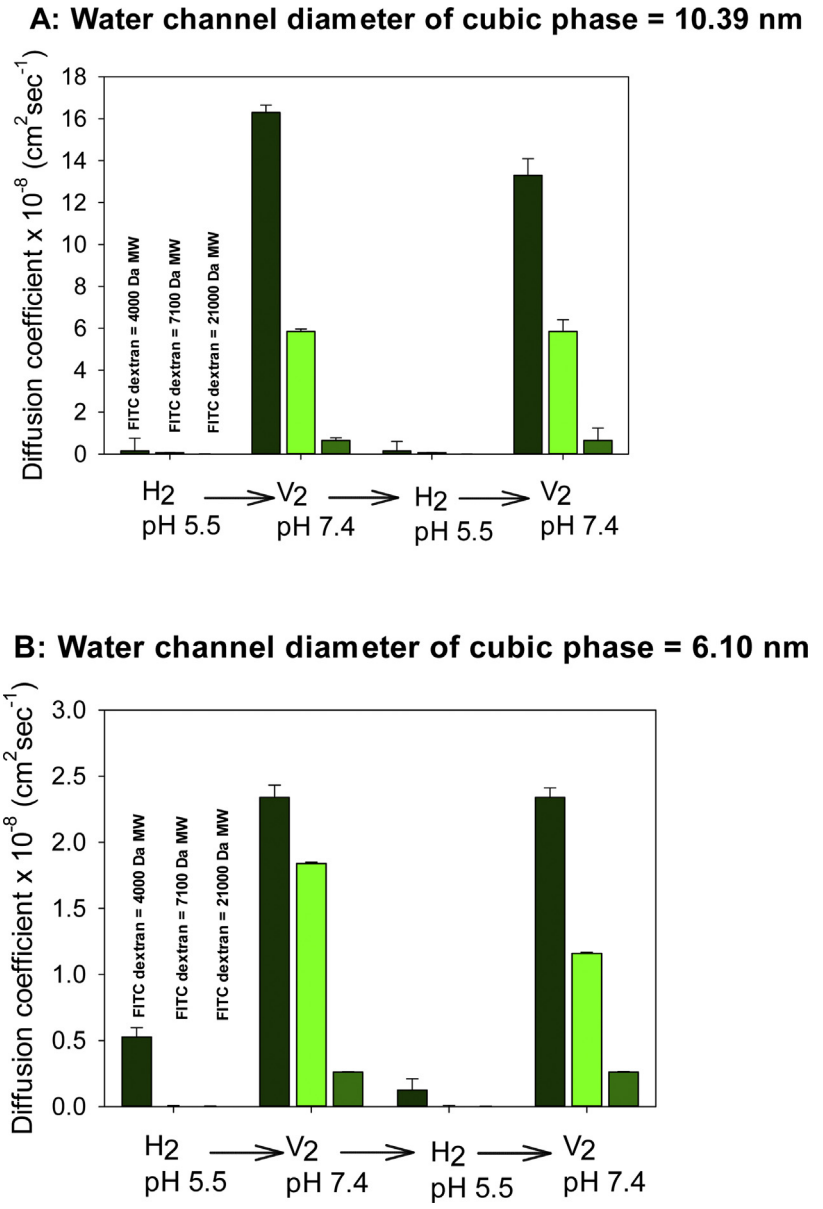
A: Water channel diameter of cubic phase = 10.39 nm
Diffusion coefficient x 10-8 (cm2sec-1)
FITC dextran = 4000 Da MW
FITC dextran = 7100 Da MW
FITC dextran = 21000 Da MW
H2 pH 5.5
V2 pH 7.4
H2 pH 5.5
V2 pH 7.4
B: Water channel diameter of cubic phase = 6.10 nm
Diffusion coefficient x 10-8 (cm2sec-1)
FITC dextran = 4000 Da MW
FITC dextran = 7100 Da MW
FITC dextran = 21000 Da MW
H2 pH 5.5
V2 pH 7.4
H2 pH 5.5
V2 pH 7.4

Fig. 7. The diffusion coefficients from the release study of FITC-dextran from systems with differing water channel diameters while altering the LC phases. (Data are mean ± s. d, $n = 3$).

The percentage of FITC-dextran released from the different liquid crystalline systems over time at different pHs are shown in Fig. 6. In general, at pH 5.5, when the internal structure of the LC systems was the hexagonal phase, the release rate of the FITC-dextran was very slow. When the system converted to the reverse cubic phase by switching to pH 7.4 the release rate increased dramatically. Switching back to pH 5.5 again reduced the release rate, which again increased when the pH was increased back to 7.4. The change in release rate with alternating pH indicates that the pH-triggered release behaviour is reversible, similar to that of temperature-triggered release behaviour in previous studies (Ho et al., 1972).

In Panel A, where the water channel size was the largest out of the two liquid crystal systems, 10.39 nm, a minimal amount of dextran was released in the initial H_2 phase. Upon the first phase conversion to V_2 phase approximately 50%, 20% and 10% of the total 4000, 7100 and 21,000 Da FITC-dextrans were released respectively consistent with the trends in size. In Panel B, where smaller water channels were prevalent for the cubic phase, 6.10 nm, a similar pattern was evident whereby the majority of FITC-dextran release occurred in the V_2 phase. However, expectedly when compared to the larger water channel cubic phase system, a reduced total amount of FITC-dextran was released.

All systems studied showed linear release behaviour with square root of time that was modelled using the Higuchi diffusion model and the calculated diffusion constants are summarized in Fig.7. The 'on–off' nature of the diffusion on changing pH is clear in this mode of illustration.

4. Discussion

In recent years, lipid-based liquid crystalline systems have been generating interest for their potential use as drug nanocarriers for a variety of compounds (Boyd, 2003; Boyd et al., 2009, 2007; Dong et al., 2011, 2006, 2010). This is due to the highly ordered internal structure of water channels, which leads to the possibility of controlled and sustained release for incorporated molecules of varying size and polarity. Phases of particular interest that have

been widely studied and characterised in the literature include cubic and hexagonal (Phan et al., 2011). However, due to the small size of the channels the molecules need to diffuse out, larger macromolecules have previously shown slow or non-existent release. The channel diameters for unmodified typical systems are of the order of 2-4 nm, closely matched to the size of small macromolecules (Erickson, 2009).

It is well known that modifying lipid packing can alter phase structure of the liquid crystal mesophases. The most common way of doing this is through external factors such as pH, ionic strength, temperature, pressure and the presence of additives. This study involved investigating systems that altered the mesophase and induced phase switching in response to changes in additives and pH. The pH responsive release of the hydrophilic drug phloroglucinol from cubic and hexagonal mesophases has previously been investigated in GMO and linoleic acid systems, although at a single composition without a focus on swelling of the cubic phase (Negrini and Mezzenga, 2011). A similar molecule to linoleic acid, oleic acid, was used in the current study. Oleic acid is protonated at an acidic pH and becomes deprotonated at neutral pH, causing the critical parameter to modify lipid packing, resulting in a reversible change from the reverse hexagonal phase to the reverse bicontinuous cubic phase, with release from the cubic phase being considerably faster (Du et al., 2014; Salentinig et al., 2010). Specifically, the protonated form of oleic acid is hydrophobic, residing within the hydrophobic region of the bilayer inducing an increase in the critical packing parameter driving the system towards reverse hexagonal phase structure. On ionization at the higher pH, the oleic acid occupied the interface, with charge–charge repulsion inducing swelling of the water channels.

The scattering patterns using SAXS of all formulations containing oleic acid confirmed that at pH 5.5 the reverse hexagonal (H_2) phase was present, while at pH 7.4 the bicontinuous cubic (V_2) phase was present. The inclusion of sucrose did not alter this behaviour. Negrini and Mezzenga (2012) have shown that the inclusion of ST, a hydration-modulating additive, induced mesophase swelling in the V_2 phase. The studies conducted in this paper confirmed these findings and additionally investigated the behaviour of OA and ST in combination.

When comparing the lattice parameter and size of the water channels for systems containing PHYT alone and PHYT + OA compared to those with the inclusion of ST it is clear the ST significantly increases the lattice parameter of the cubic phase and therefore water channel size. Formulations with PHYT + 0–25% w/w OA and no ST were tested and the lattice parameter and diameter of water channel ranged from 75 Å to 123 Å and 3.69 nm to 6.63 nm respectively, this shows that OA alone does induce mesophase swelling. However, inclusion of ST created an additive effect to again increase the lattice parameter and water channel diameter with the largest water channels seen with 15% OA and 30% ST with a lattice parameter of 185 Å and water channel diameter of 10.39 nm.

Drug release from V_2 phase and H_2 phases has been well established and it is known that release from the bicontinuous cubic phase with generally larger open water channels is more rapid than hexagonal phase (Fong et al., 2009, 2014; Lee et al., 2009). Phan et al. studied the release of glucose, a small molecule, from the bicontinuous cubic phase and hexagonal phase and showed diffusion coefficients of $D = 3.6 \times 10^{-8}\,cm^2\,s^{-1}$ and $D = 1.7 \times 10^{-8}\,cm^2\,s^{-1}$ respectively (Phan et al., 2011). The findings of the PHYT/OA/ST systems in this study were consistent with these previous studies and highlighted that both phases are controlled by first-order diffusion, indicated by the linear relationship with diffusion and square root of time.

Clogston and Caffrey (2005) illustrated that the larger the macromolecule the slower its diffusion within the matrix. Lee et al. (2009) investigated the release of 20,000 Da FITC-dextran from V_2 phase containing PHYT alone and after 14 days release was too slow to enable measurement. In the current study, release of 21,000 Da FITC-dextran was achieved, albeit slow but it was still detectable ($D = 2.60 \times 10^{-9}\,cm^2\,s^{-1}$ and $D = 6.90 \times 10^{-9}\,cm^2\,s^{-1}$ for the small and large water channel sizes respectively). A reason for the faster diffusion coefficients in this study can be attributed to the inclusion of sucrose stearate and oleic acid. The larger water channels induced by ST allow for faster release of larger molecules than previously seen.

In this study the diffusion coefficients of FITC-dextran are different for the V_2 phase during the first switch and the second switch. For the 4000 Da FITC-dextran in the V_2 phase with a water channel size of 10.39 nm the diffusion coefficient measured after the first switch to cubic phase was $D = 16.3 \times 10^{-8}\,cm^2\,s^{-1}$, however after being switched to the H_2 phase and again back to the V_2 phase, the diffusion coefficient of the V_2 phase was lower at $13.3 \times 10^{-8}\ cm^2\,s^{-1}$. This could be attributed to change in the structure over time and the cubic phase not fully equilibrating during the second switch. A similar difference was obtained during analogous studies with direct heating using glucose (Fong et al., 2009); further in situ SAXS studies during the pH switching event are planned to better understand this phenomenon.

5. Conclusion

It was shown that the inclusion of sucrose stearate and oleic acid provided a synergistic effect on swelling the cubic phase at neutral pH, whereas at low pH the oleic acid worked against the effect of sucrose stearate due to its incorporation into the hydrophobic regions of the hexagonal phase structure. The ability to use pH as an external trigger to switch between minimal drug release of a macromolecule from the hexagonal phase with closed smaller water channels to greater drug release from the cubic phase with more open water channels was demonstrated for different sized model macromolecules. This research is promising for future therapeutic use in molecules including DNA, antibodies and proteins where impractically slow release kinetics may have hindered development, and also in controlling burst release from dispersed liquid crystalline systems which will be the subject of future studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2015.08.072.

References

Boyd, B.J., 2003. Characterisation of drug release from cubosomes using the pressure ultrafiltration method. Int. J. Pharm. 260, 239–247.
Boyd, B.J., Dong, Y.-D., Rades, T., 2009. Nonlamellar liquid crystalline nanostructured particles: advances in materials and structure determination. J. Liposome Res. 19, 12–28.
Boyd, B.J., Rizwan, S.B., Dong, Y.D., Hook, S., Rades, T., 2007. Self-assembled geometric liquid-crystalline nanoparticles imaged in three dimensions:

hexosomes are not necessarily flat hexagonal prisms. Langmuir 23, 12461–12464.
Briggs, J., Chung, H., Caffrey, M., 1996. The temperature-composition phase diagram and mesophase structure characterization of the monoolein/water system. J. Phys. II 6, 723–751.
Clogston, J., Caffrey, M., 2005. Controlling release from the lipidic cubic phase. Amino acids, peptides, proteins and nucleic acids. J. Control. Release 107, 97–111.
Dong, Y.-D., Larson, I., Bames, T.J., Prestidge, C.A., Boyd, B.J., 2011. Adsorption of nonlamellar nanostructured liquid-crystalline particles to biorelevant surfaces for improved delivery of bioactive compounds. ACS Appl. Mater. Interfaces 3, 1771–1780.
Dong, Y.-D., Larson, I., Hanley, T., Boyd, B.J., 2006. Bulk and dispersed aqueous phase behavior of phytantriol: effect of vitamin E acetate and F127 polymer on liquid crystal nanostructure. Langmuir 22, 9512–9518.
Dong, Y.-D., Tilley, A.J., Larson, I., Lawrence, M.J., Amenitsch, H., Rappolt, M., Hanley, T., Boyd, B.J., 2010. Nonequilibrium effects in self-assembled mesophase materials: unexpected supercooling effects for cubosomes and hexosomes. Langmuir 26, 9000–9010.
Du, J.D., Liu, Q.T., Salentinig, S., Nguyen, T.H., Boyd, B.J., 2014. A novel approach to enhance the mucoadhesion of lipid drug nanocarriers for improved drug delivery to the buccal mucosa. Int. J. Pharm. 471, 358–365.
Erickson, H.P., 2009. Size and shape of protein molecules at the nanometer level determined by sedimentation, gel filtration, and electron microscopy. Biol. Proced. Online 11, 32–51.
Fong, W.-K., Hanley, T., Boyd, B.J., 2009. Stimuli responsive liquid crystals provide 'on-demand' drug delivery in vitro and in vivo. J. Control. Release 135, 218–226.
Fong, W.-K., Hanley, T.L., Thierry, B., Tilley, A., Kirby, N., Waddington, L.J., Boyd, B.J., 2014. Understanding the photothermal heating effect in non-lamellar liquid crystalline systems, and the design of new mixed lipid systems for photothermal on-demand drug delivery. Phys. Chem. Chem. Phys. 16, 24936–24953.
Ho, N.F.H., Higuchi, W.I., Shipman, C., Turi, J., 1972. Systems approach to study of drug transport across membranes using suspension cultures of mammalian—cells. 1. Theorectical diffusion models. J. Theor. Biol. 34 451-&.
Hyde, S., 2012. Identification of lyotropic liquid crystalline mesophases. In: Holmberg, K. (Ed.), Handbook of Applied Surfaces and Colloid Chemistry. John Wiley & Sons pp. 299–332.
Israelachvili, J., 1994. The science and applications of emulsions—an overview. Colloids Surf. A: Physicochem. Eng. Aspects 91, 1–8.
Kaasgaard, T., Drummond, C.J., 2006. Ordered 2-D and 3-D nanostructured amphiphile self-assembly materials stable in excess solvent. Phys. Chem. Chem. Phys. 8, 4957–4975.
Kirby, N.M., Mudie, S.T., Hawley, A.M., Cookson, D.J., Mertens, H.D.T., Cowieson, N., Samardzic-Boban, V., 2013. A low-background-intensity focusing small-angle X-ray scattering undulator beamline. J. Appl. Crystallogr. 46, 1670–1680.
Kost, J., Langer, R., 2012. Responsive polymeric delivery systems. Adv. Drug Deliv. Rev. 64, 327–341.
Langer, R., 1990. New methods of drug delivery. Science 249, 1527–1533.
Lee, K.W.Y., Nguyen, T.-H., Hanley, T., Boyd, B.J., 2009. Nanostructure of liquid crystalline matrix determines in vitro sustained release and in vivo oral absorption kinetics for hydrophilic model drugs. Int. J. Pharm. 365, 190–199.
Mulet, X., Boyd, B.J., Drummond, C.J., 2013. Advances in drug delivery and medical imaging using colloidal lyotropic liquid crystalline dispersions. J. Colloid Interface Sci. 393, 1–20.
Negrini, R., Mezzenga, R., 2011. pH-responsive lyotropic liquid crystals for controlled drug delivery. Langmuir 27, 5296–5303.
Negrini, R., Mezzenga, R., 2012. Diffusion, molecular separation, and drug delivery from lipid mesophases with tunable water channels. Langmuir 28, 16455–16462.
Phan, S., Fong, W.-K., Kirby, N., Hanley, T., Boyd, B.J., 2011. Evaluating the link between self-assembled mesophase structure and drug release. Int. J. Pharm. 421, 176–182.
Salentinig, S., Sagalowicz, L., Glatter, O., 2010. Self-assembled structures and pK(a) value of oleic acid in systems of biological relevance. Langmuir 26, 11670–11679.
Yaghmur, A., Glatter, O., 2009. Characterization and potential applications of nanostructured aqueous dispersions. Adv. Colloid Interface Sci. 147–48, 333–342.
Zabara, A., Mezzenga, R., 2014. Controlling molecular transport and sustained drug release in lipid-based liquid crystalline mesophases. J. Control. Release 188, 31–43.